

“Sexual” behavior in parthenogenetic lizards (*Cnemidophorus*)

(evolution/endocrinology)

DAVID CREWS* AND KEVIN T. FITZGERALD†

*Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138; and †Department of Environmental, Population and Organismal Biology, University of Colorado, Boulder, Colorado 80309

Communicated by Edward O. Wilson, October 31, 1979

ABSTRACT All-female, parthenogenetic species afford a unique test of hypotheses regarding the nature and evolution of sexuality. Basic data on the behavior of parthenogens are lacking, however. We have discovered, from observations of captive *Cnemidophorus uniparens*, *C. velox*, and *C. tesselatus*, behavior patterns remarkably similar to the courtship and copulatory behavior of closely related sexual species. Briefly, in separately housed pairs, one lizard was repeatedly seen to mount and ride its cagemate and appose the cloacal regions. Dissection or palpation revealed that, in each instance, the courted animal was reproductively active, having ovaries containing large, preovulatory follicles, while the courting animal was either reproductively inactive or postovulatory, having ovaries containing only small, undeveloped follicles. These observations are significant for the questions they raise. For example, is this behavior a nonfunctional vestige of the species' ancestry, or is this behavior necessary for successful reproduction in the species (e.g., by priming reproductive neuroendocrine mechanisms as has been demonstrated in sexual species)?

All-female, parthenogenetic species present a unique opportunity to test hypotheses regarding the nature and evolution of sexuality. Whereas the majority of vertebrates are gonochoristic (i.e., separation of the sexes in different individuals), at least 27 species of reptiles are known to consist mostly or entirely of females and to reproduce only clones of female offspring (1). The most thoroughly studied lizard parthenogens belong to the genera *Cnemidophorus* and *Lacerta*, and much is now known concerning the cytogenetics and ecology of several species (2, 3). Little information, however, is available regarding the behavior of these animals (4, 5). Such information would provide valuable insight into the role of behavior in the neuroendocrine control of reproductive processes in parthenogenetic species. Presented here are observations of captive parthenogenetic lizards exhibiting behavior patterns remarkably similar to the courtship and copulatory behavior of sexual congeners.

Members of the parthenogenetic species *Cnemidophorus uniparens* were collected in Cochise County, Arizona in June 1978 and 1979. The initial observations were made on two pairs of healthy, sexually mature lizards maintained in large aquaria. In late November 1978, intense social activity was noted in the cages, and daily observations were initiated. In June 1979, additional animals ($n = 57$) were collected from the same area and housed in one of three social conditions: groups of four, pairs, or isolates (J. Gustafson and D. Crews, unpublished data). In July and August 1979, six complete pseudocopulations were observed; irregular observations indicate that the occurrence of this behavior is not rare. These observations confirmed in all respects the findings of the previous year and so are combined here.

Shortly after emergence on each day of observation, one animal (e.g., animal 2 of pair A and animal 4 of pair B) would

begin to lunge at and bite its cagemate. Attacks were always initiated by the same individual. Bites were directed initially toward the whole body, but soon concentrated on the tail. The attacked animal would react at first defensively and attempt to bite back, but this behavior rapidly decreased with the lizard becoming progressively more passive. This was followed by extended periods of malelike courtship behavior in which the formerly aggressive individual would grip with its jaws the tail or leg of its cagemate, after which she would mount the back of the now passive female. The mounting female would ride atop the mounted female for as long as 2 min (Fig. 1). During this time, she would intermittently rub her cloaca against the dorsal pelvic area of the mountee, stroking the back and neck with her jaws and forelimbs. The active lizard would then grasp the back of the neck or some region of the shoulder of the other female in its jaws and begin to curve its tail beneath the other's tail so as to appose the cloacal regions. In one pseudocopulation the mounting female was seen to evert partially her cloacal region. (In sexual *Cnemidophorus* species, the male achieves intromission with one of a pair of hemipenes everted through the cloaca.) In both pairs of parthenogenetic females, cloacal contact was immediately followed by the courting female quickly shifting her jaw grip to the posterior third of the mounted female's body, thereby forming the contorted mating posture characteristic of this genus (unpublished observations; see also refs. 7–12). Females maintained this posture for 1.0–5.0 min ($\bar{x} = 2.32$ min, range 1–4 min, $n = 4$). Concurrent observations of the sexual behavior of *C. tigris*, and reports of the courtship and mating in *C. inornatus* (see ref. 12), both closely related sexual species, revealed behavior patterns remarkably similar to those described above. It is significant to note that at no time were any females of sexual *Cnemidophorus* species housed in our laboratories observed to mount one another.

Dissection of the first three *C. uniparens* revealed that the mounted animal in each pair was reproductively active, having ovaries containing large vitellogenic (i.e., yolky) follicles (Table 1). On the basis of Cuellar's (5) estimate that ovulation occurs in this species when the ovarian follicles are 8.0–9.0 mm in diameter, these females were due to ovulate within 3–4 days. On the other hand, the dissected active or “courting” female was found to be reproductively inactive, having ovaries containing only small, previtellogenic (i.e., nonyolky) follicles measuring 1.2–2.2 mm in diameter. In the original observations, the one female not sacrificed at the time of the observations (female 4, Table 1), oviposited five eggs 25 days after exhibiting mounting behavior. Cuellar (5) has determined that the interval between vitellogenesis and oviposition in this species is 20–23 days. Thus, it is likely that this female also contained previtellogenic follicles and was reproductively inactive at the time the behavior was observed. Palpation of six other pairs in which complete sequences of malelike sexual behavior was observed yielded similar results.

In another parthenogenetic species, *Cnemidophorus velox* (a triploid all-female species believed to be derived from *C.*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U. S. C. §1734 solely to indicate this fact.

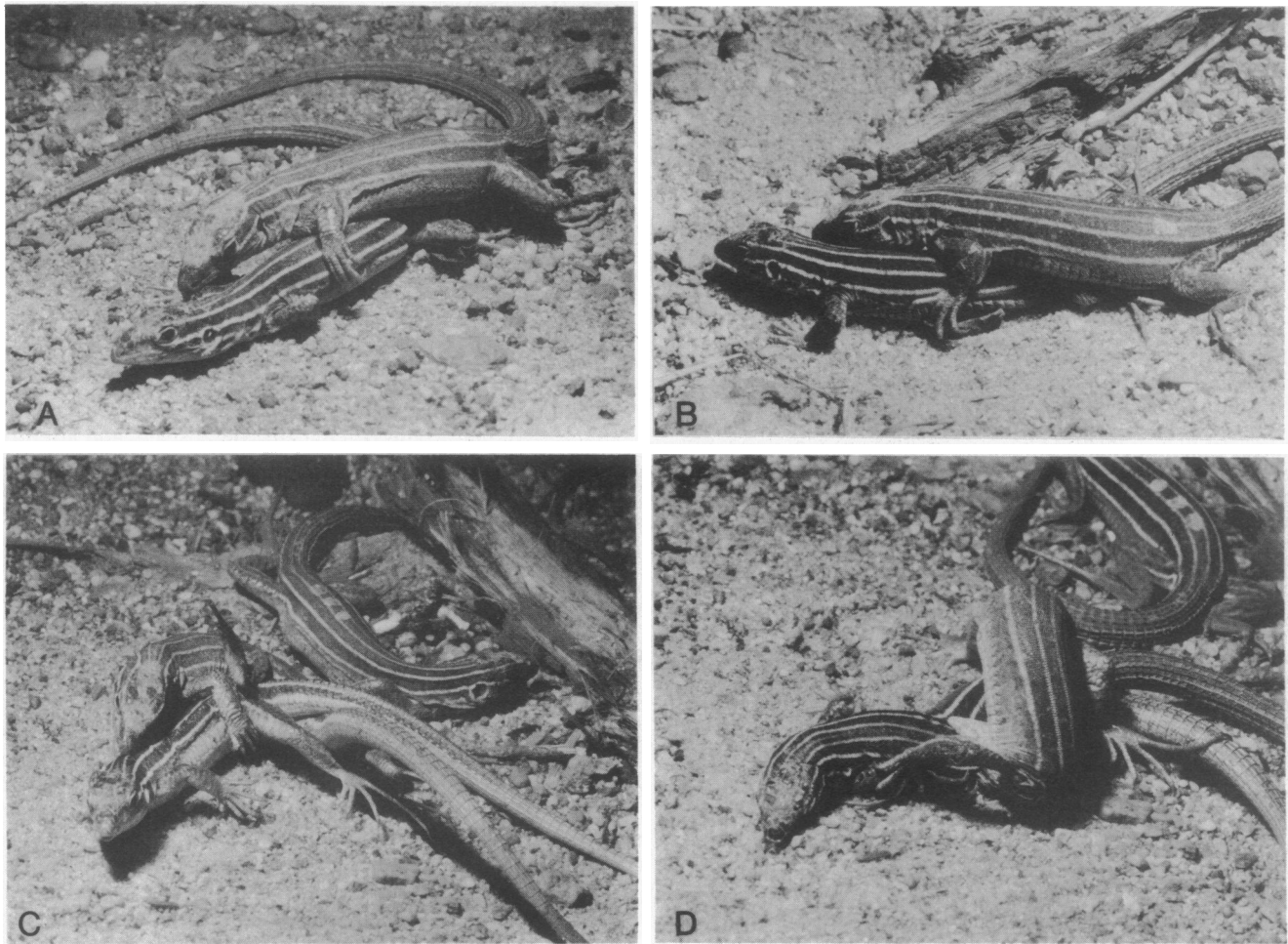


FIG. 1. "Sexual" behavior in captive parthenogenetic *Cnemidophorus uniparens*. After lunging attacks directed at the smaller female, the larger female approaches the now passive small female, first gripping in her jaws the foreleg (A). This is accompanied by mounting and riding behavior (A, B), during which the active female scratches the side of the mounted female with her fore- and hind-legs and strokes the back of her neck with her jaw. Shortly afterwards, the active female twists her tail beneath the other's tail (C), apposing the cloacae and assuming the copulatory posture characteristic of sexual cnemidophorine lizards (D). Females were housed in pairs or groups in aquaria measuring 76.2 × 30.5 cm. Heat was provided by a 75-W, 120-V lamp suspended 10 cm from the sand substrate. A water bowl was provided at the opposite end of the cage. Each cage was illuminated by two Durotest Vita lights 30 cm above the cage bottom. A 14-hr dark:10-hr light cycle was employed, with a daily temperature gradient of 25°C near the water dish and 47°C directly under the heat lamp. The temperature dropped to 21°C at night. Lizards were fed both mealworms and crickets ad lib. Further details of care and maintenance procedures of parthenogenetic *Cnemidophorus* are provided in ref. 6.

inornatus and one or more other unknown ancestors of the *C. sexlineatus* group), female–female mountings were also observed. The lizards were collected near Clifton, Mesa County, Colorado in early August 1977. In the laboratory, the animals were housed in pairs and observed. Sociosexual behavior was observed in 3 of 15 *C. velox* pairs that strikingly resembled the courtship and mating behavior seen in *C. inornatus* also housed in the laboratory and the copulatory behavior described previously for other sexual cnemidophorine species (7–12). In *C. velox*, mounting behavior was initiated by the courting animal as a series of bites and nips directed toward the hind limbs and tail of the other lizard. If the other animal did not flee, the aggressive lizard would seize with its jaws either the tail, a hind limb, or the skin on the back of the more passive animal, and subsequently try to maneuver its own body until mounting was accomplished. Once in this riding position, the bite-hold was transferred toward the head, with the mounting animal pressing the other lizard into the sand, holding the now passive animal in place. If no further resistance was encountered, the mounting lizard next attempted to force its tail under that of the quiescent lizard to appose the vents. In each instance the female remained

mounted for approximately 3 min; mounting was terminated when the quiescent animal made good an escape. Upon dissection of these animals, the courting lizards were found to have ovaries containing only small nonyolking follicles, whereas the mounted animals possessed between four and six large, conspicuously yolking follicles (Table 1). It is significant that only in these three pairs were females found to be preovulatory; dissection of the other 24 females revealed all to have previtellogenic follicles.

A female–female mounting was also observed in a pair of captive *C. tessellatus*, another unisexual member of this genus, collected in July 1977 near Pueblo, Colorado. The more active female of this pair pursued the other around the cage, accentuating the chase with frequent and vigorous bites at the tail of the fleeing lizard. Once the tail was seized, the aggressive lizard quickly shifted the bite to the back and mounted the now passive lizard, pressing the body of the quiescent animal into the substrate. The mounting lizard then forced her tail beneath the tail of the passive lizard, thereby bringing the cloacal regions into contact. The mounting lizard stayed in the copulation posture for nearly 4 min; the encounter ended when the passive

Table 1. Reproductive condition of three species of parthenogenetic lizards (*Cnemidophorus uniparens*, *C. velox*, and *C. tessellatus*) at time of observations

Pair	Animal	Behavior	Snout-vent length, mm	Ovarian condition and size of largest follicles, mm	Number of follicles
<i>Cnemidophorus uniparens</i>					
A	1	Femalelike	68	Preovulatory (4.8–5.4)	7*
	2	Malelike	59	Previtellogenic (1.2–2.2)	7*
B	3	Femalelike	57	Preovulatory (6.0–6.2)	7*
	4	Malelike	67	Previtellogenic	5†
C	5	Femalelike	60	Preovulatory	3†
	6	Malelike	56	Postovulatory	0†
D	7	Femalelike	67	Preovulatory	4†
	8	Malelike	56	Postovulatory	0†
E	9	Femalelike individual not identified			
	10	Malelike	65	Postovulatory	2†
F	11	Femalelike	66	Preovulatory	3†
	12	Malelike	57	Postovulatory	2†
G	13	Femalelike	63	Preovulatory	2†
	14	Malelike	57	Postovulatory	2†
H	15	Femalelike	66	Preovulatory (≥6.0)	3‡
	16	Malelike	68	Postovulatory (≤3.0)	3‡
I	17	Femalelike	69	Preovulatory (≥6.0)	3‡
	18	Malelike	71	Postovulatory (≤3.0)	2‡
J	19	Femalelike	65	Preovulatory (≥6.0)	3‡
	20	Malelike	71	Postovulatory (≤3.0)	2‡
K	21	Femalelike	66	Preovulatory (≥6.0)	3‡
	22	Malelike	71	Postovulatory (≤3.0)	2‡
L	23	Femalelike	66	Preovulatory (≥6.0)	3‡
	24	Malelike	72	Postovulatory (≤3.0)	2‡
M	25	Femalelike	66	Preovulatory (≥6.0)	3‡
	26	Malelike	71	Postovulatory (≤3.0)	3‡
<i>Cnemidophorus velox</i>					
A	1	Femalelike	58	Preovulatory (6.5–7.0)	4*
	2	Malelike	67	Previtellogenic (1.2–2.0)	7*
B	3	Femalelike	69	Preovulatory (6.5–7.5)	6*
	4	Malelike	55	Previtellogenic (1.2–1.4)	6*
C	5	Femalelike	66	Preovulatory (6.4–7.0)	5*
	6	Malelike	63	Previtellogenic (0.8–1.0)	5*
<i>Cnemidophorus tessellatus</i>					
A	1	Femalelike	75	Preovulatory (7.5–8.0)	5*
	2	Malelike	69	Previtellogenic (1.2–2.0)	8*

Female reproductive state was determined by dissection at the time of the observations, egg-laying records, or palpation as noted.

* Determined by immediate dissection.

† Estimate of reproductive condition based on egg-laying record or, in the instance of females 6 and 8, on change in body weight.

‡ Estimate of ovarian condition based on palpation; estimate of number of follicles based on number of eggs subsequently laid.

lizard suddenly freed herself. Dissection of the animals once again revealed that the ovaries of the active lizard possessed no yolking follicles, whereas the ovaries of the courted animal contained five large preovulatory follicles (Table 1).

Successful matings between parthenogens and sexual congeners have been well documented both in the field and under laboratory conditions (12, 13). Furthermore, naturally occurring hybrids of several sexual and parthenogenetic *Cnemidophorus* species have been reported (12, 14–16). However, our observations presented here are the first to document apparent "sexual" behavior between parthenogenetic individuals. It is noteworthy that in 9 years of observations of captive populations of the iguanid lizard, *Anolis carolinensis*, intact females have on only four occasions been observed to court and mount other females; in each instance, the females that exhibited malelike behavior have been found to be reproductively inactive and the courted animal, to be preovulatory. Recent experiments have

demonstrated that ovariectomized *A. carolinensis* treated with testosterone will court and mount other ovariectomized females that have been estrogen-primed (17). Finally, it is unlikely that this masculine behavior is an age-dependent phenomenon as in some sequentially hermaphroditic fish (18). In half of the pairs observed in which both individuals could be identified ($n = 16$), the courting lizard was the smaller (younger) of the two, whereas, in the other pairs, the larger (older) female mounted the smaller female.

It is likely that social interactions play an important role in the reproductive biology of parthenogenetic *Cnemidophorus* because these species are found in high densities in the southwestern United States (see ref. 3). In addition, it is well known that in gonochoristic vertebrates male sexual behavior primes neuroendocrine mechanisms regulating ovarian activity (19–21). Is this malelike behavior in parthenogenetic *Cnemidophorus* simply a nonfunctional vestige of the species' sexual

ancestry? Or, is it necessary for successful reproduction and thus compensates for the evolutionary loss of male-related stimuli critical to normal ovarian functioning? Recent experiments reveal that females mounted on the same day oviposit simultaneously. Is the hormonal state of the mounting female different from that of the female being courted? The finding that only preovulatory females are mounted and only previtellogenic or postovulatory females mount would indicate this to be an important variable. Can this malelike behavior, complete with pseudocopulation, be elicited by hormone manipulation? We have found that intact, reproductively inactive *C. uniparens* mount and copulate with preovulatory females upon implantation of silastic pellets of testosterone or dihydrotestosterone, but not estrogen. Thus, as in gonochoristic vertebrates, malelike sexual behavior in parthenogenetic *Cnemidophorus* may be required for successful reproduction.

We thank R. R. Tokarz, C. S. Carter, E. E. Williams, E. O. Wilson, D. McClearn, and an anonymous reviewer for suggestions on the manuscript. We also thank Elizabeth Gordon for collecting the *Cnemidophorus uniparens* in 1978 and Brian Camazine and Jill Gustafson for their assistance in the field in 1979. Special thanks go to Jill Gustafson for caring for the *C. uniparens* in the laboratory. This work was supported in part by National Science Foundation Grant BNS 75-13796, National Institute of Child Health and Human Development Grant HD 12709, and National Institute of Mental Health Research Scientist Development Award 1 KOZ MH 00135 (to D.C.) and by a Biomedical Grant from the University of Colorado Graduate School.

1. Cole, C. J. (1975) in *Intersexuality in the Animal Kingdom*, ed. Reinboth, R. (Springer, New York), pp. 340-355.
2. Cuellar, O. (1977) *Science* **197**, 837-843.
3. Mitchell, J. C. (1979) *Can. J. Zool.* **57**, 1487-1499.
4. Milstead, W. W. (1967) *Tex. J. Sci.* **9**, 410-447.
5. Cuellar, O. (1971) *J. Morphol.* **133**, 139-166.
6. Cole, C. J. & Townsend, C. R. (1977) *Experientia* **33**, 285-288.
7. Fitch, H. S. (1958) *Univ. Kans. Mus. Nat. Hist. Misc. Publ.* **11**, 11-62.
8. Carpenter, C. C. (1962) *Am. Midl. Nat.* **67**, 132-151.
9. Hardy, D. F. (1962) *Univ. Kans. Sci. Bull.* **43**, 1-73.
10. Marhdt, C. R. (1976) *Southwest Nat.* **21**, 252-254.
11. Noble, G. K. & Bradley, H. T. (1933) *Ann. N.Y. Acad. Sci.* **35**, 25-100.
12. Neaves, W. B. (1971) *Breviora*, No. 381, 1-25.
13. Fitzgerald, K. T. (1978) *J. Colo.-Wyo. Acad. Sci.* **10**, 5 (abstr.).
14. Cuellar, O. & McKinney, C. O. (1976) *J. Exp. Zool.* **196**, 341-350.
15. Lowe, C. H., Wright, J. W., Cole, C. J. & Bezy, R. L. (1970) *Syst. Zool.* **19**, 114-127.
16. Wright, J. W. & Lowe, C. H. (1967) *Am. Mus. Novit.* **2286**, 1-36.
17. Adkins, E. K. & Schlesinger, L. (1980) *Horm. Behav.*, in press.
18. Choat, J. H. & Robertson, D. R. (1975) in *Intersexuality in the Animal Kingdom*, ed. Reinboth, R. (Springer, New York), pp. 263-283.
19. Adler, N. T. (1974) in *Reproductive Behavior*, eds. Montagna, W. & Sadler, W. A. (Plenum, New York), pp. 259-286.
20. Cheng, M.-F. (1978) in *Advances in the Study of Behavior*, eds. Rosenblatt, J. S., Hinde, R. A., Beer, C. G. & Busnel, M. C. (Academic, New York), pp. 97-130.
21. Crews, D. (1975) *Science* **189**, 1059-1065.